PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

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OCT 26 2005

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	28.10.2005
Applicant's or agent's file reference: 55197-017WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/024881	29.07.2004	16.10.2003

Applicant: BAYER HEALTHCARE LLC et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
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Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 55197-017WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US2004/024881	International filing date *(day/month/year)* 29.07.2004	Priority date *(day/month/year)* 16.10.2003
International Patent Classification (IPC) or national classification and IPC C07K16/38, G01N33/53		
Applicant BAYER HEALTHCARE LLC et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.08.2005	28.10.2005

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/024881

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-5, 7-9, 12-14, 17-26	as originally filed
6, 10, 11, 15, 16	received on 15.08.2005 with letter of 12.08.2005

Claims, Numbers

1-32	received on 15.08.2005 with letter of 12.08.2005

Drawings, Sheets

1/3-3/3	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☒ the description, pages 6,10,15,16
   - ☒ the claims, Nos. 2,6,8,13,16,17
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/024881

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-5,7,10,11,14,15,18,19,22-24,26-32
	No:	Claims	1,9,12,20,21,25
Inventive step (IS)	Yes:	Claims	3-5,7,10,11,14,15,18,19,22-24,26-32
	No:	Claims	1,9,12,20,21,25
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No. PCT/US2004/024881

The application concerns the provision of monoclonal antibodies against the class of proteins known as urinary trypsin inhibitors (UTIs) together with immunoassays employing the same.

Re Item I

Basis of the report

The amendments filed with the letter dated 15/08/2005 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. Significant changes such as changing the composition of an immunogen used to generate antibodies or redefining the specificity of antibodies for a group of related antigens are considered to constitute amendments that go beyond the teaching of the application as filed. The amendments concerned are the following:

(i) **Claims 2, 13**: The ratio of immunogens given in the claims goes beyond the disclosure of the application as filed.

(ii) **Claims 6, 8, 16 and 17**: The affinities of the antibodies are redefined in the claims and thus extend the teaching of the application beyond the initial disclosure.

(iii) **Page 6**: The description of the affinities of the antibodies has been changed, supposedly to reflect the data of Tables 3 and 4. However, with regard to the affinity of 421-3G5 for bikunin, Tables 3 and 4 are in disagreement with each other; according to Table 3 there is no interaction between this mAb and bikuinin. Any amendment of the teaching of the application regarding the affinity of specific antibodies for certain antigens extends the teaching.

(iv) **Page 10**: The ratios of proteins in the immunogen composition has been changed. There is no basis in the description for this change.

(v) **Page 15**: Changing the words "even though" to "as" changes the teaching of the application beyond the scope of the original disclosure.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 Reference is made to the following documents:

D1: TREFZ G ET AL: "ESTABLISHMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR URINARY TRYPSIN INHIBITOR BY USING A MONOCLONAL ANTIBODY" JOURNAL OF IMMUNOASSAY, MARCEL DEKKER, BASEL, CH, vol. 12, no. 3, 1991, pages 347-369, XP009008646 ISSN: 0197-1522

D2: US-B1-6 242 197 (PAPUASHVILI MARINA N) 5 June 2001 (2001-06-05)

D3: KOBAYASHI HIROSHI ET AL: "Identity of urinary trypsin inhibitor-binding protein to link protein" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 28, 14 July 2000 (2000-07-14), pages 21185-21191, XP002322618 ISSN: 0021-9258

D4: US 2003/190732 A1 (JOSIC DJURO) 9 October 2003 (2003-10-09)

D5: PUGIA MICHAEL J ET AL: "Clinical utility of a rapid test for uristatin" CLINICAL BIOCHEMISTRY, vol. 35, no. 2, March 2002 (2002-03), pages 105-110, XP002322619 ISSN: 0009-9120

V.2 **Novelty - Art.33(1) and (2) PCT:**

i Documents D1-D4 all disclose monoclonal antibodies raised against UTIs. D1 and D2 further disclose immunoassays employing such antibodies for the detection of UTIs in biological fluids, most notably in urine and plasma or serum. The subject matter of **claims 1, 9, 12, 20, 21 and 25** is therefore considered to be anticipated by the prior art.

ii The specific monoclonal antibodies and technical features of subject matter disclosed in **claims 3-5, 7, 10, 11, 14, 15, 18, 19, 22-24 and 26-32** appear to be novel over the cited prior art.

V.3 **Inventive Step - Art.33(1) and (3) PCT:**

i The closest prior art can be taken to be document D1 or D2, which both disclose monoclonal antibodies and immunoassays against certain UTIs (uristatin 1 and 2 in the case of D1; total UTI in D2). The provision of further antibodies can only be considered to be inventive if they result in an as yet undisclosed technical effect. For example, claims directed toward methods differentiating between different UTIs (**claims 24 and 27**) would appear to be inventive.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/US2004/024881

Re Item VII
Certain defects in the international observation

VII.1 The Application provides three distinct monoclonal antibodies with differing specificities for the various species of UTI (421-5G8, which appears to bind to uristatin 1 and 2 with a higher affinity than to bikunin or uristatin; 420-5D11, which appears to bind to THP with a higher affinity than to bikunin or uristatin and 421-3G5, which binds all tested UTIs but not Pαl or Iαl). It appears that arriving at antibodies with the 'preferential' affinities is a matter of trial and error, presenting the skilled person with an undue burden in arriving at the claimed products. There is no clear instruction in the application leading the skilled person to directly arrive at antibodies with specific preferences, and thus an objection is raised according to Article 5 PCT for the subject matter of **claims 4 and 15**, as said subject matter is not considered to be sufficiently disclosed for the skilled person to arrive at the given antibodies or methods.

VII.2 **Claims 25, 27 and 28** directed to methods for directly measuring the concentration of specific UTIs further requires antibodies which do not just prefer one UTI over another, but bind specifically and exclusively to the different constituent proteins in order to allow the desired specific or differential assay. The application does not disclose such antibodies, rather it provides antibodies which *prefer* certain UTI species. The subject matter of the given claims is therefore not properly disclosed or supported (Articles 5 and 6 PCT).

VII.3 In contrast to the requirements of Rule 13*bis*.3 PCT, the application does not disclose the information stipulated in part (a) items (i)-(iv) for the three named monoclonal antibodies.

Re Item VIII
Certain observations on the international observation

VIII.1 Claims 1 and 12 are incorrect from a scientific standpoint and therefore lack clarity.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/US2004/024881

The claims relate to a monoclonal antibody "secreted by a hybridoma produced from antibodies". Hybridomas are not produced from antibodies but the other way round (Article 6 PCT).

common proteins in urine and serum such as Human Serum Albumin (HSA), Tamm-Horsfall protein (THP), α-1-microglobulin (α-1M), α-1-antichymotrypsin (α-1ACT) and non-inhibitory urinary trypsin inhibitors (pro-inhibitor forms) such as I-α-I and P-α-I.

Direct measurement of a UTI may be obtained by either using antibodies that recognize only that particular UTI. Indirect measurement of a UTI may be made by subtraction using antibodies that recognize UTIs other than the one of interest and antibodies that recognize all or most of the prevalent UTIs. In the examples below, antibodies secreted by hybridoma ATCC 421-3G5.4C5.3B6 and hybridoma ATCC 421-5G8.1A8.5C1 are shown to be suitable for determining the amount of UTIs in both urine and blood while hybridoma ATCC 420-5D11.5G8.1E4 is suitable for correction of cross reactivity and determining the amount of UTIs. Other antibodies will be shown to have similar characteristics.

Such monoclonal antibodies may be made by introducing purified UTIs into mice as an immunogen. Single hybridoma clones producing only one antibody have been created by carrying out the procedure of Kohler and Milstein using purified uristatin. The characteristic properties of the monoclonal antibodies have been studied, using the Surface Enhanced Laser Desorption/Ionzation (SELDI) technique, ELISA, and other immunoassay methods.

In another aspect, the invention includes methods of using the novel monoclonal antibodies to detect and measure forms of urinary trypsin inhibitors of interest, that is, those characterizing persons having disease. Where a monoclonal antibody is specific to an inhibitory UTI, e.g. to Bikunin, and Uristatin, or to the precursor AMBK, they can be measured directly. When a monoclonal antibody is able to bind to more than one UTI, then by using more than one antibody, the content of a particular UTI of interest may be determined by difference. The three monoclonal antibodies referred to above have been found to bind preferentially to Uristatin and Uristatin-1 and -2 Mab 420-5D11 binds strongly also to the Tamm-Horsfall protein (THP) and less strongly to bikunin and AMBK. Mab 421-3G5 also binds strongly to bikunin and AMBK. Mab 421-5G8 also binds less strongly to bikunin.

Analyses may be carried out on samples of many biological fluids, including but not limited to blood, urine, water, saliva, spinal fluid, intestinal fluid, food, and blood plasma. Blood and urine are of particular interest.

Brief Description of the Drawings

Fig. 1 is a bar chart of the results of Example 5.

ELISA (enzyme-linked immunosorbent assay) provides a very sensitive method for detecting antigens. In an antigen down ELISA, a microtiter plate receives a sample suspected of containing a certain antigen. After allowing for adsorption of the antigen onto the plate, and washing off all non-bound materials, the monoclonal antibody is added, incubated so that it can bind to the antigen and the excess washed off. The added monoclonal antibody may have already been labeled with a reporter molecule to permit the generation of a signal to be read by any number of techniques. Alternatively a ligand capable of attaching to the antibody (e.g. an anti-mouse antibody conjugated to a reporter molecule) is added. After excess of the enzyme-coupled ligand has been washed off, the chromogen or other substrate is added if necessary and the color developed used as an indicator of the amount of antigen present.

There are various immunoassay methods that could be used to apply antibodies of the invention such as microparticle capture immunoassays (MIC), latex agglutination inhibition (LAI), solid phase chromatographic (IC), radioimmunoassays (RIA), enzyme linked immunosorbent assays (ELISA), enzyme linked assays (EIA), fluorescence linked assays (FIA), luminescence linked assays (LIA), rare earth metals label assays, chemiluminescence assays (CLA) and optical color label assays (OA) such as colored latex particle and colloidal gold. It is also feasible to use electrochemical signal transducers (EST) based on amperometric, impedimetric, and potentimetric detection methods.

Example 1

Preparation of Monoclonal Antibodies

BALB/c mice were immunized with 100 µg/mouse of purified UTIs obtained from renal patients by SciPac Ltd. Sittingbourne, Kent, UK, product code P250-1 to produce a response. The immunogen composition as determined by SDS-PAGE contained about > 85% of the material 17kDa (uristatin), plus > 10% uristatin 1 or 2 and < 5% of bikunin (30.9 kDa) and no detectable AMBK, I-α-I, or P-α-I. After one month, ocular bleeds were taken from each mouse and titered by ELISA against the immunogen to assess the immune response. The mice showing the best response were boosted by injection of 100 µg/mouse with the immunogen. After four days, mice were sacrificed and their spleens used for fusion according to the method of Kohler and Milstein, Nature 256:495 (1975). The splenocytes were fused with SP2-0 Ag14 myeloma cells using PEG (polyethylene glycol) solution with a ratio of splenocytes to Myeloma cells of 5:1 and plated into 96 well plates using 50%

PEG/HAT growth media. After 7-10 days of incubation at 37 degrees Celsius, fusion cultures were monitored for growth by feeding every 3-4 days utilizing the HAT (hypoxanthine, aminopterin, thymidine) selection method followed by subculturing with HAT growth media.

After 2-3 weeks, the wells having hybridoma colony growth were tested by ELISA to determine which growths produced an antibody immune response to the purified uristatin peptide used to innoculate the mice. The 96 well plate cultures were tested with the uristatin peptide at 1ug/mL coated plates. After coating plates overnight at 2-8°C, all plates were washed and blocked. Cell culture supernatants were then applied 100µl/well for one hour at room temperature. After washing plates, Goat anti-mouse IgG Horse Radish Peroxidase at 1:2000 dilution was applied at 100uL/well for one hour. Plates were washed once again followed by OPD (o-phenylene diamine dihydrochloride) substrate and read at 490nm on a Spectra Max plate reader.

The colonies giving a positive response were transferred to 24 well plates for further expansion and retesting to verify the positive results. The colonies testing positive were further expanded in six well plates in Iscove's Modified Dulbecco's Medium (IMDM) with 10% Fetal Bovine Serum (FBS). After expansion, the colonies were frozen at - 70°C and then transferred to liquid nitrogen for long-term storage.

Based on ELISA results using the purified UTIs, various clones were further expanded in IMDM, 10% FBS and frozen down.

Example 2

Screening Procedure of Polyclonal Antibodies

Rabbit polyclonal antibodies raised against the purified UTIs were used in the screening process as this antibody was expected to be non-specific for any given form of UTIs. Serum and urine specimens from three patients with infection and two healthy controls were characterized by western blot tests using these polyclonal antibodies. The western blot tests used the commercial pre-cast gel system (Invitrogen, San Diego CA). Urine and plasma specimens were loaded with 1 µg and 5 µL per lane. The western blots were stained with a WesternBreeze® chromogenic immunodetection kit (from Invitrogen) following the manufacturer's instructions. Rabbit anti-UTI antiserum was used at a dilution of 1:250 000 as the primary antibody in the western blot analysis (See Table 1). These western blot results demonstrated that the rabbit polyclonal antiserum

The urine samples for the controls were assayed by the ELISA assay described in Example 1 using the three monoclonal antibodies selected in Example 2 and a Goat Anti-Mouse secondary antibody conjugate. The results for group A clone 421 – 3G5, group B clone 421 – 5G8 and group C clone 420 – 5D11 are shown in Table 2 and compared to the polyclonal antibody as a control.

The measurements made of color developed by the OPD substrate indicated the amount of the monoclonal antibody bound to UTIs in the standards just described, whose contents were determined by molecular weights using the western blot method and SELDI. The values were normalized using the polyclonal antibody values and are presented in the table below as percent relative to the polyclonal antibody results.

Table 2. ELISA Results with Specimens from Patient and Healthy Individual and Protein Standards for UTIs.

Specimen	2-12/17/35/ 60-80 kDa %	ANTIBODY GROUP B CLONE 421 - 5G8	ANTIBODY GROUP C CLONE 420 – 5D11	ANTIBODY GROUP A CLONE 421 – 3G5	POLYCLONAL ANTIBODY
UTI Standard lot 20-120	10/15/45/30	92%	100%	71%	100%
UTI Standard lot 124-111	10/80/10/0	100%	13%	100%	94%
UTI Standard lot 80-117	0/100/0/0	5%	0%	48%	74%

The monoclonal antibody 421-5G8 bound strongly to UTI lots #124-111 and #20-120 to a similar degree, but only bound weakly to Lot #80-117. This would be consistent with binding to the 2-12 kDa material (Uristatin 1 or 2) in both lots but not in lot 80-117 containing only the 17 kDa material (Uristatin). Thus, this antibody appears specific for Uristatin 1 or 2 over Bikunin and Uristatin. While not wanting to be limited to a mechanism, it is believed that binding by this monoclonal antibody does not occur through the sulfated chondroitin chain even though these moieties are known to possess high antigen affinities for antibodies. The binding is thought to be direct to an inhibitory amino acid sequence.

The monoclonal antibody 420-5D11 bound strongly to UTI lot #20-120, very weakly to lot #124-111, and did not bind to lot 80-117. This is consistent with binding to

the 80 kDa material (THP) in lot #20-120, since only lot #20-120 contains a large amount of this material. If the antibody were specific for Uristatin or Bikunin also, one would expect it to have strongly responded to lot #124-111, which contained about 65% of Uristatin. This antibody appears to bind to THP but also to Bikunin and Uristatin. While not wanting to be limited to a mechanism, it is believed that binding by this monoclonal antibody does not occur through a common amino acid region between THP and Bikunin or Uristatin. Sequence matching has not shown enough homology in the Uristatin 1 and 2 domains and THP for this to be possible.

The monoclonal antibody 421 – 3G5 bound strongly to all UTI lots and similar to the non-specific polyclonal antibody with the notable exception of lacking reactivity to P-α-I and I-α-I. Thus 421-3G5 would be a measure of total UTI.

Example 4

Testing of Monoclonal Antibodies with Clinical Specimens

Urine samples were obtained from patients having bacterial infections and from control patients having no such infections. The patients without infections are the "group 1" patients. To be included in the study, we only required a negative urine and blood culture (10^5 organisms/mL) and a normal blood white blood count (CBC). The second group of patients ("group 2") included those with infections either upper respiratory tract or urinary tract, a conclusion based on a positive complete blood count (CBC) in all. Clean-catch midstream urine collections were obtained from all patients and controls. Specimens were stored at 4°C until tested; but if not tested within 24 hours, storage was at –70 °C until tested. For all subjects, the evaluation of urine sediment, gram stain and urine microbiological culture were always performed on the day of collection. We collected EDTA-anticoagulated blood from group 2 and performed a CBC, a high sensitivity CRP (Dade Behring, Immunoassay for C-reactive protein) test, and a blood culture and performed all these tests on the day of blood collection.

The urine samples were also assayed by an ELISA assay using the same polyclonal antibodies as in Example 2. The antibodies were immobilized in polystyrene membrane wells of the high binding microtiter plates (PN 3690 Corning Life Sciences, Acton, MA), then wells were coated with Super Block (Pierce Chem Co., Rockford IL) to ensure none of the following additions attach directly to the plate, then contacted with

WHAT IS CLAIMED IS: (96)

1. A monoclonal antibody for detecting urinary trypsin inhibitors (UTI) in the biological fluids of persons having disease, said monoclonal antibody being secreted by a hybridoma produced from antibodies to purified uristatin.

2. A monoclonal antibody of Claim 1 secreted by a hybridoma produced from purified uristatin containing greater than 85% uristatin, about 10% uristatin 1 or 2, and less than 5% bikunin .

3. A monoclonal antibody of Claim 2, wherein said antibody being secreted by hybridoma ATCC 421-5G8.1A8.5C1.

4. A monoclonal antibody of Claim 3, wherein said UTI are uristatin and uristatin-1 and -2.

5. A monoclonal antibody of Claim 2, said antibody being secreted by hybridoma ATCC 420-5D11.5G8.1E4.

6. A monoclonal antibody of Claim 5, wherein said UTI are uristatin, uristatin-1 and -2, Bikunin, AMBK, and said monoclonal antibody further binds to Tamm-Horsfall protein (THP).

7. A monoclonal antibody of Claim 2, said antibody being secreted by hybridoma ATCC 421-3G5.4C5.3B6.

8. A monoclonal antibody of Claim 7, wherein said UTI are uristatin, uristatin-1 and -2, and AMBK.

9. A monoclonal antibody of any one of Claims 1-8 for detecting UTI in blood or urine.

10. A monoclonal antibody of Claims 3 or 7 for detecting UTI in urine.

11. A monoclonal antibody of Claim 5 for detecting UTI in blood plasma.

12. A method of assaying a biological fluid for urinary trypsin inhibitors (UTI) comprising contacting a sample of biological fluid with a monoclonal antibody secreted by a hybridoma produced from antibodies to purified uristatin.

13. A method of Claim 12, wherein said purified uristatin contains greater than 85% uristatin, about 10 % uristatin 1 or 2, and less than 5% bikunin.

14. A method of Claim 13 , wherein said monoclonal antibody is secreted by one of the group of hybridomas ATCC 421-5G8.1A8.5C1, ATCC 420-5D11.5G8.1E4, and ATCC 421-3G5.4C5.3B6 and identifying UTIs bound to said antibody.

15. A method of Claim 14, wherein said monoclonal antibody is secreted from hybridoma ATCC 421-5G8.1A8.5C1 and said UTIs are uristatin and uristatin-1 and -2.

16. A method of Claim 14, wherein said monoclonal antibody is secreted from hybridoma ATCC 420-5D11.5G8.1E4 and said UTIs are uristatin, uristatin-1 and -2, Bikunin, and AMBK and said monoclonal antibody further binds to Tamm-Horsfall protein (THP).

17. A method of Claim 14, wherein said monoclonal antibody is secreted from hybridoma ATCC 421-3G5.4C5.3B6 and said UTIs are uristatin, uristatin-1 and 2, and AMBK.

18. A method of Claim 14 wherein said UTIs are identified in an immunoassay.

19. A method of Claim 18 wherein said immunoassay is selected from the group consisting of MIC, LAI, IC, RIA, ELISA, EIA, FIA, LIA, CLA, OA, EST and rare earth metals label assays, as defined herein.

20. A method of Claim 12 wherein said biological fluid is urine.

21. A method of Claim 12, wherein said biological fluid is blood plasma.

22. A method of Claim 20, wherein said antibody is secreted by the hybridoma ATCC 421-5G8.1A8.5C1 or hybridoma ATCC 421-3G5.4C5.3B6.

23. A method of Claim 21, wherein said antibody is secreted by the hybridoma ATCC 420-5D11.5G8.1E4.

24. A method of Claim 12, wherein all UTIs are measured by a first monoclonal antibody and specific UTIs measured by a second antibody are subtracted from the UTIs measured by the first antibody to measure the UTIs found by the first antibody, but not by the second antibody.

25. A method of Claim 12, wherein specific UTIs are measured directly by an antibody secreted by one of said hybridoma.

26. A method of assaying a biological fluid for urinary trypsin inhibitors (UTI) comprising the steps of:

(a) adding a biological fluid sample suspected of containing urinary trypsin inhibitors to a substrate;

(b) adding to said sample of (a) a monoclonal antibody secreted from a hybridoma selected from the group consisting of ATCC 421-5G8.1A8.5C1, ATCC 420-5D11.5G8.1E4, and ATCC 421-3G5.4C5.3B6;

(c) adding to the combined monoclonal antibody of (b) and the biological sample of (a) a ligand capable of binding to said monoclonal antibodies, said ligands being bound to an enzyme;

(d) washing from the combined monoclonal antibody of (b), the biological sample of (a), and the ligand of (c) the portion of said ligand unbound to said monoclonal antibody;

(e) determining the amount of said urinary trypsin inhibitors bound to said monoclonal antibody and said ligands by adding a reported molecule capable of developing a signal by reaction with said enzyme; and

(f) correlating the signal developed with the amount of said urinary trypsin inhibitors.

27. A method of Claim 26, wherein all UTIs are measured by a first monoclonal antibody and specific UTIs measured by a second antibody are subtracted from the UTIs measured by the first antibody to measure the UTIs found by the first antibody but not by the second antibody.

28. A method of Claim 26, wherein specific UTIs are measured directly by an antibody secreted by one of said hybridomas.

29. A method of Claim 26, wherein said biological sample is urine.

30. A method of Claim 29, wherein said antibody is secreted by the hybridoma ATCC 421-5G8.1A8.5C1, or hybridoma ATCC 421-3G5.4C5.3B6.

31. A method of Claim 26, wherein said biological sample is blood plasma.

32. A method of Claim 31 wherein said antibody is secreted by the hybridoma ATCC 420-5D11.5G8.1E4.